

**STUDIES ON IMPACT OF ETIOLATION AND PLANT GROWTH
REGULATORS CONCENTRATION ON ROOTING BEHAVIOUR OF
AIR LAYERS OF GUAVA (*Psidiumguajava* L.)**

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ABSTRACT

The experiment was conducted in the horticulture garden at Bihar Agricultural college, Sabour in year 2009-2010.. The design of experiment was Randomized Block Design with three replications. The experiment comprised of twelve treatments including control. The symbol and treatment detail is are as follows ;- T_1 - Control, T_2 - Etiolation, T_3 - NAA @ 3000 ppm, T_4 - NAA @ 6000 ppm, T_5 - NAA @ 3000 ppm + etiolation, T_6 - IBA @ 3000 ppm, T_7 - IBA@6000 ppm, T_8 - IBA @ 3000 ppm + etiolation, T_9 - NAA @ 3000 ppm + IBA @ 3000 ppm, T_{10} - NAA@ 3000 ppm + IBA @ 6000 ppm, T_{11} - NAA @ 6000 ppm + IBA @ 3000 ppm and T_{12} - NAA @ 6000 ppm + IBA @ 6000 ppm. Maximum rooting(94.66 %) was obtained with etiolation followed by application of IBA @ 3000 ppm concentration, which was statistically at par with T_5 (91.33 %) and T_7 (88.33 %). Whereas, control i.e., T_1 showed 54.33 per cent rooting in air-layers.Etiolation followed by application of growth regulators i.e., IBA @ 3000 ppm showed the maximum number and length of secondary roots i.e., 15.75 & 11.62 cm. The maximum fresh weight(2.90 g), dry weight(0.94 g) and number(18.33) of new leaves were recorded with etiolation + IBA @ 3000 ppm concentration. The best treatment in respect of survivability was T_8 (etiolation + IBA @ 3000 ppm) which showed maximum survival(78.33 %). Next effective treatment was T_5 (etiolation + NAA @ 3000 ppm) with 75.90 per cent survival of air-layers in nursery.

KEYWORDS: Etiolation, IBA, NAA, Concentration and Rooting Behaviour

Original Article

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INTRODUCTION

Guava(*Psidiumguajava* L.) is the fourth most common and popular fruit of India in area and production after mango, citrus and banana. It has been in cultivation in India since 17th century and gradually became a crop of commercial importance. Guava belongs to the family Myrtaceae and native of tropical America. It is most important, highly productive, delicious and nutritious fruit of tropical and sub-tropical region. It is said to be the “apple of tropics”(Hayes, 1957). It is good source of Calcium, Iron, fair source of Phosphorus and a rich source of vitamin C and pectin. Guava is available at cheaper rate and is popularly known as “Poor man apple”. The vitamin ‘C’ content of the fresh ripe fruit varies from 100 to 260 mg per 100g of fruit pulp. Beside this, it is also a good source of vitamin A, vitamin B and minerals. Guava has an important place in the fruit industries because it provides a good raw material for making jelly and many kinds of other preserves and does not loss the vitamin C content in preserved form. It is also reputed for its medicinal properties and almost all the parts of plants are used in medicines(Kirtkar and Basu, 1950).

Guava can be propagated by air-layering, inarching, stooling, root cutting and budding. Air-layering is the most popular commercial method of vegetative propagation of guava in Bihar. The success and survival of the plants are poor. Air-layering was evaluated as a commercial method of vegetative propagation of guava (Albany *et al.*, 2004).. The success of air-layering depends on factors like varying conditions of climate, species and varieties of the plant and environmental factors like air, temperature, humidity and mechanical treatments. Physiological condition of mother plant, age of wood and season during which cutting are taken also affect the rooting of cuttings. Internal and structural factors like stored food material in the layerage, maturity of the tissues, etiolation and callusing. With the advancement of horticultural science, the technology of air-layering has also been improved.

Pre conditioning treatments such as girdling, blanching and etiolation of shoots have been shown to induce roots in some difficult to-root fruit plants. Etiolation stimulates the rooting at etiolated portion. Etiolation means, the development of plant parts in absence of light. This results in loss of green colour in etiolated portions.

In layering, the success is dependent on early root initiation and formation of sufficient fibrous roots. Regeneration of roots in air-layers is largely controlled by a number of external and internal factors. Etiolation stimulates the rooting at etiolated portion and has resulted in better rooting in a number of fruit plants. It is now a well-known fact that etiolation treatment increases the temporary accumulation of endogenous growth substances in the etiolated portion due to some anatomical abnormality, which promotes better rooting and quality of roots(Mukherjee and Chatterjee, 1978 and Dhua and Sen, 1984).

In the recent past the use of plant growth regulators have played a key role in various phases of plant growth and development. It has also influenced in early root initiation and in producing fibrous roots. These two are limiting factors which effects on the success of propagated layers. A number of plant growth substances have been used successfully, in rooting of cuttings and layering. The most commonly used plant growth regulators are IBA, NAA and IAA. However, the selection of growth regulators and their concentrations varies with fruit plants.

While using growth regulators, use of proper concentration is also important factor because growth regulators used in excessive concentration may results in injury and cause yellowing and dropping of leaves, blackening of stem and eventual death of cutting and layering, usually a concentration just below the toxic point is considered the most favourable for root formation, while lower concentration may inhibit the growth, higher concentration may become toxic and this range of action is very narrow. Therefore, from standardization point of view, it is essential to determine the suitable concentration with etiolation in plant physiology by way of experimentation.

Keeping all the facts under consideration and visualizing the paucity of information on these aspects, the present investigation was undertaken with objective to see the efficiency of etiolation and plant growth regulators in inducing roots and growth of air layers of guava.

MATERIALS AND METHODS

The present investigation was carried out during the year 2009-10 to study the impact of etiolation and plant growth regulators i.e., IBA and NAA on survival, regeneration of roots, rooting ability, growth and development of layers in the nursery to air-layering of guava. The experiment was conducted with twelve treatment replicated thrice in the monsoon season. The experiment was conducted in the Horticulture Garden of Bihar Agricultural College, Sabour(Bhagalpur). For the present investigation seven years old guava plant of uniform growth was selected as

experimental plant. The plants were healthy, free from pests and diseases and were grown under similar agro-climatic condition. Similar cultural and manurial schedules were adopted. One or two years old shoots having the pencil thickness(i.e., approximately 0.8 to 1.0 cm) were selected for air-layering. Twenty shoots were selected for each treatment. The selected shoots, which have to be ringed, the 4-6 cm long shoot portion wrapped with a band of black polythene film. The etiolation was done before one month of layering. The root study was undertaken in the air-layers after detachment from the mother plant. In this process, before taking observations the media particles adhering to the roots were washed out carefully with fine water spray without damaging the root system. The excess water adhering to the roots was dried by means of a blotting paper. The numbers of rooted layers were counted after 70 days of layering. The percentage of rooted layers was computed in relation to total number of layers tied. The number of primary roots per layer were noted or counted in washed samples for each treatment and replication separately and average was calculated.

The number of secondary roots per primary roots was counted in washed samples for each treatment and replication separately and average was computed. The diameter of primary roots was measured with the help of slide calipers. After taking fresh weight, the roots were dried in a hot oven at 80°C for 24 hours then dry weight was obtained per layer.Ultimate survival and mortality of air-layers in the nursery were recorded after 180 days of detachment and percentage of survival for each treatment was computed.

RESULTS

The results of different characters i.e. rooting characters in relation to the percentage of rooted air-layers, the number of secondary roots, diameter of roots, fresh and dry weight of roots of air-layers were recorded at 70 days of layering. Establishment of air-layers in the nursery, survival percentage of air-layers, average number of leaves per layer and diameter of new shoots were recorded after 180 days of detachment from the mother plants and finally the data were analysed and interpreted.Observations on rooting percentage of air-layers revealed that success in air-layering induced by all etiolation followed by application of growth regulators, varied from 54.33 to 94.66 per cent. The maximum rooting percentage was recorded in T₈(94.66%) which was statistically at par with the treatments T₅(91.33%) and T₇(88.33%). Whereas, the minimum rooting percentage was found in T₁(54.33%) followed by treatment T₂ which was statistically at par with T₄.Among all the treatments, etiolation followed by application of IBA @ 3000 ppm was found most effective, followed by etiolation with application of NAA @ 3000 ppm concentration. While, with growth regulators NAA @ 6000 ppm concentrations showed minimum effect on the success of rooted layers(71.66%). Guava layers which treated with etiolation followed by application of IBA @ 3000 ppm concentration recorded maximum number(15.75) of secondary roots, which was closely related to etiolation followed by application of NAA @ 3000 ppm concentration. NAA @ 6000 ppm + IBA @ 6000 ppm concentration ranked third(13.50) which was closely associated with T₉(13.00) and T₇(12.75). The observations on the diameter of roots revealed that the maximum diameter of roots was in treatment T₈(0.23 cm), which was statistically at par with T₅(0.22 cm) and T₁₂(0.22 cm), were significantly differ from minimum diameter was recorded in T₁(0.15 cm), which was statistically equal to T₂(0.16 cm) with respect to etiolation followed by application of growth regulator treatments.

Table 1: Average Rooting Success of Air-Layers in Per Cent (70 Days after Layering)

Treatmens	Chemicals and concentrations	Rooting(%)	Number of Secondary Roots	Diameter of Roots	Fresh Weight of Root(g)	Dry Weight of Roots(g)
T ₁	Control	54.33	5.66	0.15	1.91	0.63
T ₂	Etiolation	66.33	9.50	0.16	1.94	0.64
T ₃	NAA @ 3000 ppm	78.33	9.66	0.18	2.05	0.68
T ₄	NAA @ 6000 ppm	71.66	8.25	0.17	1.98	0.66
T ₅	NAA @ 3000 ppm + Etiolation	91.33	14.50	0.22	2.82	0.94
T ₆	IBA @ 3000 ppm	84.66	11.25	0.18	2.15	0.69
T ₇	IBA @ 6000 ppm	88.33	12.75	0.19	2.18	0.70
T ₈	IBA @ 3000 ppm + Etiolation	94.66	15.75	0.23	2.90	0.96
T ₉	NAA @ 3000 ppm + IBA @ 3000 ppm	85.00	13.00	0.21	2.26	0.73
T ₁₀	NAA @ 3000 ppm + IBA @ 6000 ppm	82.66	11.66	0.20	2.23	0.72
T ₁₁	NAA @ 6000 ppm + IBA @ 3000 ppm	79.33	10.33	0.18	2.20	0.71
T ₁₂	NAA @ 6000 ppm + IBA @ 6000 ppm	86.66	13.50	0.22	2.34	0.74
SEm±		2.78	0.49	0.0065	0.0821	0.0251
C D at 5 %		8.17	1.46	0.0191	0.2410	0.0737

It is evident from the table-1 that fresh weight of roots per layer varied from 1.91 g(T₁) to 2.90 g(T₈). The best treatment in respect of fresh weight was T₈(2.90 g) which was at par with T₅(2.82 g). The dry weight of roots was maximum in T₈(0.96 g) followed by T₅(0.948), T₁₂(0.74 g) and T₉(0.73 g), respectively. However, the minimum dry weight of roots per layer was recorded in T₁(0.63 g) i.e. control. Among the treatment, etiolation without application of growth regulators showed lesser dry weight of roots 0.64 g in T₂.

The survival and mortality of air-layers in the nursery was recorded at 180 days after the detachment of layers from mother plants in respect of total number of layers prepared at the time of layering. The percentage of survival was recorded in Table-2. Etiolation followed by application of IBA @ 3000 ppm was found most effective in increasing the survival of air-layers. Etiolation followed by application of NAA @ 3000 ppm concentration recorded second; whereas IBA @ 6000 ppm concentration was obtained third position. It was observed that the number of new leaves per layer varied from 9.33 to 18.33 in different treatments. Maximum number of new leaves was observed in T₈(18.33) which was statistically at par with T₅ (17.66), whereas minimum number of leaves found in T₉(9.57) i.e. Control, which was statistically similar to T₃(10.66). On critical examination of the table-2 it was revealed that the maximum diameter (2.60 cm) was recorded in T₈, closely followed by T₅(2.50 cm) whereas the minimum diameter of new shoots was found in T₁(1.70 cm) i.e., control followed by T₂(1.80 cm) and T₃(1.80 g).

Table 2: Average Survival of Air-Layers (%) (180 Days after Detachment)

Treatmens	Chemicals and Concentrations	Survival (%)	Number of Leaves/Air Layer	Diameter of New Shoots(cm)
T ₁	Control	50.33	9.33	1.70
T ₂	Etiolation	61.00	11.66	1.80
T ₃	NAA @ 3000 ppm	64.75	10.66	1.90
T ₄	NAA @ 6000 ppm	62.27	12.33	1.95
T ₅	NAA @ 3000 ppm + Etiolation	75.90	17.66	2.50
T ₆	IBA @ 3000 ppm	74.30	12.66	2.05

Table 2: Contd.,				
T ₇	IBA @ 6000 ppm	71.42	13.33	2.13
T ₈	IBA @ 3000 ppm + Etiolation	78.33	18.33	2.60
T ₉	NAA @ 3000 ppm + IBA @ 3000 ppm	68.15	13.66	2.08
T ₁₀	NAA @ 3000 ppm + IBA @ 6000 ppm	66.38	15.00	2.19
T ₁₁	NAA @ 6000 ppm + IBA @ 3000 ppm	64.25	14.00	2.12
T ₁₂	NAA @ 6000 ppm + IBA @ 6000 ppm	69.82	16.66	2.26
SEM±		1.82	0.4847	0.082
C D at 5 %		5.35	1.4214	0.2582

DISCUSSIONS

Guava is usually propagated by vegetative means and air-layering is the commercial practice among nurserymen. In layering, success depends on early root initiation and formation of sufficient fibrous roots. Etiolation stimulates the rooting at etiolated portion in the number of fruit plants. Recently growth regulators are being used to achieve more success in rooting. Among different growth regulators, auxins like IBA and NAA are found to be more effective. While using growth regulators, use of proper concentration is also important because excessive dose may cause yellowing and dropping of leaves, blackening of stem and eventually death of layers, while lower concentration may inhibit the growth. Etiolation of shoot cleaved before air-layering definitely have profound effect on rooting ability and root quality of air-layers. The etiolated region accumulated more sugar natural auxin and other rooting co-factors, which are known to increase adventitious root formation. Etiolation treatment of shoot further improved the regeneration capacity of layers. Etiolation may reduce the production of lignin, thus instead of forming lignin phenolic metabolites may be channeled to enhance root initiation. Different scientists observed that the application of root promoting substances(iba, NAA and IAA etc) used in etiolated shoots gave higher percentage of success than non-etiolated shoots. Mukherjee and Bid (1965) revealed that etiolated shoots of mango treated with IBA @ 10,000 ppm and NAA @ 5000 ppm concentration induced 100 per cent rooting. Bid and Mukherjee(1969) reported that 100 per cent rooting was found in Langra mango with the concentration of 10,000 ppm of IBA or 5000 ppm of IBA+NAA in etiolated layered shoots. Chausa variety of mango gave 98.66 per cent rooting with etiolated shoots treated with IBA+NAA @ 5,000 ppm, whereas, in Bombay green variety complete success was found in etiolated shoots when treated with IBA @ 5,000 ppm.

Observation on rooting percentage revealed that the success in air-layering induced by etiolation followed by application of growth regulators varied from 54.33 to 94.66 per cent. The maximum rooting percentage was recorded in T₈(94.66 %) which was statistically at par with the treatments T₅(91.33%) and T₇(88.33%). Whereas, the minimum rooting percentage was found in T₁(54.33%) i.e., control. Rooting of air-layers was affected by etiolation and type of growth regulators used. IBA was found most effective in regeneration of roots in the air-layers of guava in comparison to NAA. IBA proved superior because of their greater chemical stability and low mobility which improved persistance and correspondingly more prolonged action, giving better chance of success. The compound was also retained near its point of application and did not spread to other parts of cutting and induced other responses(Audus, 1963). Mukherjee and Chatterjee(1978) recorded 100 per cent rooting in jackfruit with the application of IBA @ 10000 ppm to etiolated shoots. They have also observed that IBA produced maximum rooting with higher percentage of survival. Sharma *et al.* (1980) revealed that 90-100 per cent rooting with IBA @ 200 ppm in guava air-layers. IBA also reduced the time for root initiation from 45 to 31 days and increased number of roots per layer. Exogenous application of IBA markedly improved the regeneration of roots in comparison to NAA. Hartman and Kester(1965) opined that greater effectiveness of IBA over NAA may be due to its non-toxic nature in a wide range of concentrations and was effective in promote rooting in large

number of plant species. The significant effect of IBA over NAA was also reported by Tyagi and Patel (2004) and Lalet *et al.*(2007).

As regards the effective of etiolation to concentration of growth substances, IBA @ 3000 ppm was found most effective, followed by NAA @ 3000 ppm concentration. It is also observed that IBA induced higher rooting percentage than NAA treatment and control. These results are in support of the findings of Pancholczoket *et al.* (2005) and Bastoset *et al.*(2009) who observed that IBA was better than NAA. In the present investigation the percentage of rooting was increased significantly in IBA @ 3000 ppm to etiolated shoot as compared to other treatments and control. These results are in agreement with the findings of Bhandoria (1985), who reported that IBA at all the levels helped in better root initiation and optimum concentration of IBA(3000 ppm) proved to be most effective concentration. The number of secondary roots varied from 5.66 to 15.75.

Maximum secondary roots were obtained under treatment etiolated shoot with IBA @ 3000 ppm followed by etiolated shoot + NAA @ 3000 ppm, respectively. Among IBA and NAA, IBA was found most effective in producing more number of primary and secondary roots. The increase in number of roots may be due to the accumulation of carbohydrates, enhanced protein synthesis and greater accumulation of rooting co-factors near the etiolated region. Knypl(1966) and Chhonkar and Yadav (1970) noted that inhibitors of RNA and protein synthesis inhibited IAA induced growth of Sunflower hypocotyls sections and reduced rooting and subsequent growth of roots. He concluded that auxin induced growth phenomenon were dependent on RNA and protein synthesis and suggested that IAA stimulates synthesis of RNA. Among auxins, IBA proved more effective probably due to its greater stability in layers.

Dry weight of roots was greatly influenced by use of IBA and NAA. Etiolated shoot with IBA @ 3000 ppm was found optimum for higher dry weight of roots. The beneficial effect of IBA on dry weight of roots were observed Chovatia and Singh(2000) and Tyagi and Patel(2004).

The entire growth regulator produced significantly more number of leaves, maximum length and diameter of shoots over control. These results are in conformity of Syamal and Singh(1993) reported that highest percentage of rooting and survival of air-layers of litchi have been successfully achieved by improved method of exogenous application of IBA @ 300 ppm after 60 days of etiolation. Highest success, average number of secondary roots, diameter of shoots and final survival were recorded with IBA @ 300 ppm.

CONCLUSIONS

Thus from the above findings it may be concluded that guava can be successfully propagated by air-layering with etiolation followed by application of plant growth regulators. The results of present investigation suggest that for maximum success and subsequent establishment of air-layers of guava in nursery, etiolation followed by application of IBA @ 3000 ppm concentration are quite useful and best.

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